

1/8

SCHEMATIC REPRESENTATION OF THE PHAGEMID VECTOR pCES1

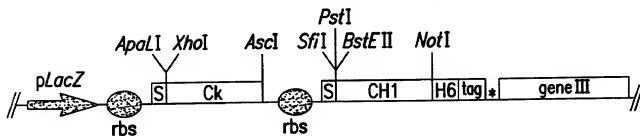


FIG. 1A

2/8

POLYLINKER REGION OF THE PHAGEMID VECTOR pCES1

Signal sequence
 ... TTA TTC GCA ATT CCT TTA GTT CCT TTC TAT TCT CAC AGT GCA CAG GTC CAA CTG CAG GTC GAC CTC GAG
 L F A I P L V V P F Y S H S A Q V Q L Q V D L E

ApalI +1
 XhoI

ATC AAA CGT GGA ACT GTG ... GGA GAG TGT TAA TAA GCG GCG CCA ATT CTA TTT CAA GGA GAC AGT CAT A
 I K R G T V G E C * *
 Human Cxk gene Stops

AscI
 rbs

Signal sequence
 ATG AAA TAC CTA TTG CCT ACG GCA GCC GCT GGA TTG TTA CTC GCG GCC CAG CCG GCG ATG GCC CAG GTG
 M K Y L L P T A A A G L L L A A Q P A M A Q V

SfiI
 +1

Signal sequence
 ATG AAA TAC CTA TTG CCT ACG GCA GCC GCT GGA TTG TTA CTC GCG GCC CAG CCG GCG ATG GCC CAG GTG
 M K Y L L P T A A A G L L L A A Q P A M A Q V

BstEII
 PstI
 CAG CTG CAG GAG AGC GGG GTC ACC GTC TCA AGC GGC TCC ACC ... AAA TCT TGT GCG GCC GCA CAT CAT CAT CAT
 Q L Q E S G V T V S S A S T K S C A A A H H H

NotI
 Hexahistidine

Human CH1 (γ1) gene

CAT CAC GGG GCG GCA GAA CAA AAA CTC ATC TCA GAA GAG GAT CTG AAT GGG GCG GCA TAG ACT GTT ...
 H H G A A E Q K L I S E E D L N G A A * I V
 Tag c-Myc Tag

Amber GeneIII

FIG.1B

Oligonucleotide primers used for construction of the library

A. Primary amplifications

IgM heavy chain constant region

HuIgMFOR 5'-TGG AAG AGG CAC GTT CTT TTC TTT-3'

κ light chain constant region

HuCKFOR 5'-ACA CTC TCC CCT GTT GAA GCT CTT-3'

λ light chain constant region

HuCI2-FOR 5'-TGA ACA TTC TGT AGG GGC CAC TG-3'

HuCI7-FOR 5'-AGA GCA TTC TGC AGG GGC CAC TG-3'

VH back

HuVH1B/7A-BACK 5'-CAG RTG CAG CTG GTG CAR TCT GG-3'

HuVH1C-BACK 5'-SAG GTC CAG CTG GTR CAG TCT GG-3'

HuVH2B-BACK 5'-CAG RTC ACC TTG AAG GAG TCT GG-3'

HuVH3B-BACK 5'-SAG GTG CAG CTG GTG GAG TCT GG-3'

HuVH3C-BACK 5'-GAG GTG CAG CTG GTG GAG WCY GG-3'

HuVH4B-BACK 5'-CAG GTG CAG CTA CAG CAG TGG GG-3'

HuVH4C-BACK 5'-CAG STG CAG CTG CAG GAG TCS GG-3'

HuVH5B-BACK 5'-GAR GTG CAG CTG GTG CAG TCT GG-3'

HuVH6A-BACK 5'-CAG GTA CAG CTG CAG CAG TCA GG-3'

B. Secondary amplifications

κ light chain constant region

HuCK-FOR-ASC

λ light chain constant region

HuCI2-FOR-ASC

HuCI7-FOR-ASC

VH back

HuVH1B/7A-BACK-SFI

HuVH1C-BACK-SFI

HuVH2B-BACK-SFI

HuVH3B-BACK-SFI

HuVH3C-BACK-SFI

HuVH4B-BACK-SFI

HuVH4C-BACK-SFI

HuVH5B-BACK-SFI

HuVH6A-BACK-SFI

FIG.2i

Oligonucleotide primers used for construction of the library

VH forward

HuJH1/2-FOR
HuJH3-FOR
HuJH4/5-FOR
HuJH6-FOR

Vκ back

HuVk1B-BACK
HuVk2-BACK
HuVk3B-BACK
HuVk4B-BACK
HuVk5-BACK
HuVk6-BACK

5'-GAC ATC CAG WTG ACC CAG TCT CC-3'
5'-GAT GTT GTG ATG ACT CAG TCT CC-3'
5'-GAA ATT GTG WTG ACR CAG TCT CC-3'
5'-GAT ATT GTG ATG ACC CAC ACT CC-3'
5'-GAA ACG ACA CTC ACG CAG TCT CC-3'
5'-GAA ATT GTG CTG ACT CAG TCT CC-3'

Vκ back

HuVk1B-BACK-APA
HuVk2-BACK-APA
HuVk3B-BACK-APA
HuVk4B-BACK-APA
HuVk5-BACK-APA
HuVk6-BACK-APA

FIG.2ii

5/8

Oligonucleotide primers used for construction of the library

V1 back		V2 back
HuV1A-BACK	5'-CAG TCT GTG CTG ACT CAG CCA CC-3'	HuV1A-BACK-APA
HuV1B-BACK	5'-CAG TCT GTG YTG ACG CAG CCG CC-3'	HuV1B-BACK-APA
HuV1C-BACK	5'-CAG TCT GTC GTG ACG CAG CCG CC-3'	HuV1C-BACK-APA
HuV2-BACK	5'-CAR TCT GCC CTG ACT CAG CCT-3'	HuV2-BACK-APA
HuV3A-BACK	5'-TCC TAT GWG CTG ACT CAG CCA CC-3'	HuV3A-BACK-APA
HuV3B-BACK	5'-TCT TCT GAG CTG ACT CAG GAC CC-3'	HuV3B-BACK-APA
HuV4-BACK	5'-CAC GTT ATA CTG ACT CAA CCG CC-3'	HuV4-BACK-APA
HuV5-BACK	5'-CAG GCT GTG CTG ACT CAG CCG TC-3'	HuV5-BACK-APA
HuV6-BACK	5'-AAT TTT ATG CTG ACT CAG CCC CA-3'	HuV6-BACK-APA
HuV7/8-BACK	5'-CAG RCT GTG GTG ACY CAG GAG CC-3'	HuV7/8-BACK-APA
HuV9-BACK	5'-CWG CCT GTG CTG ACT CAG CCM CC-3'	HuV9-BACK-APA

FIG.2iii

6/8

Oligonucleotide primers used for construction of the library

5'-ACC GCC TCC ACC GGG CGC GGC TTA TTA ACA CTC TCC CCT GTT GAA GCT CTT-3'
 5'-ACC GCC TCC ACC GGG CGC GGC TTA TTA TGA ACA TTC TGT AGG GGC CAC TG-3'
 5'-ACC GCC TCC ACC GGG CGC GGC TTA TTA AGA GCA TTC TGC AGG GGC CAC TG-3'
 5'-GTC CTC GCA ACT GCG GCC CAG CCG GCC ATG GCC CAG RTG CAG CTG GTG CAR TCT GG-3'
 5'-GTC CTC GCA ACT GCG GCC CAG CCG GCC ATG GCC SAG GTC CAG CTG GTR CAG TCT GG-3'
 5'-GTC CTC GCA ACT GCG GCC CAG CCG GCC ATG GCC CAG RTC ACC TTG AAG GAG TCT GG-3'
 5'-GTC CTC GCA ACT GCG GCC CAG CCG GCC ATG GCC SAG GTG CAG CTG GTG GAG TCT GG-3'
 5'-GTC CTC GCA ACT GCG GCC CAG CCG GCC ATG GCC GAG GTG CAG CTG GTG WCY GG-3'
 5'-GTC CTC GCA ACT GCG GCC CAG CCG GCC ATG GCC CAG GTG CAG CTA CAG CAG TGG GG-3'
 5'-GTC CTC GCA ACT GCG GCC CAG CCG GCC ATG GCC CAG STG CAG CTG CAG TCS GG-3'
 5'-GTC CTC GCA ACT GCG GCC CAG CCG GCC ATG GCC GAR GTG CAG CTG GTG CAG TCT GG-3'
 5'-GTC CTC GCA ACT GCG GCC CAG CCG GCC ATG GCC CAG GTA CAG CTG CAG TCA GG-3'

FIG.2iv

7/8

Oligonucleotide primers used for construction of the library

5'-TGA GGA GAC GGT GAC CAG GGT GCC-3'
 5'-TGA AGA GAC GGT GAC CAT TGT CCC-3'
 5'-TGA GGA GAC GGT GAC CAG GGT TCC-3'
 5'-TGA GGA GAC GGT GAC CGT GGT CCC-3'

5'-ACC GCC TCC ACC AGT GCA CTT GAC ATC CAG WTG ACC CAG TCT CC-3'
 5'-ACC GCC TCC ACC AGT GCA CTT GAT GTG ATG ACT CAG TCT CC-3'
 5'-ACC GCC TCC ACC AGT GCA CTT GAA ATT GTG WTG ACR CAG TCT CC-3'
 5'-ACC GCC TCC ACC AGT GCA CTT GAT ATT GTG ATG ACC CAC ACT CC-3'
 5'-ACC GCC TCC ACC AGT GCA CTT GAA ACG ACA CTC ACG CAG TCT CC-3'
 5'-ACC GCC TCC ACC AGT GCA CTT GAA ATT GTG CTG ACT CAG TCT CC-3'

FIG.2v

TTTTTGGGGGGG

Oligonucleotide primers used for construction of the library

5'-ACC GCC TCC ACC AGT GCA CAG TCT GTG CTG ACT CAG CCA CC-3'
 5'-ACC GCC TCC ACC AGT GCA CAG TCT GTG YTG ACG CAG CCG CC-3'
 5'-ACC GCC TCC ACC AGT GCA CAG TCT GTC GTG ACG CAG CCG CC-3'
 5'-ACC GCC TCC ACC AGT GCA CAR TCT GCC CTG ACT CAG CCT-3'
 5'-ACC GCC TCC ACC AGT GCA CTT TCC TAT GWG CTG ACT CAG CCA CC-3'
 5'-ACC GCC TCC ACC AGT GCA CTT TCT TCT GAG CTG ACT CAG GAC CC-3'
 5'-ACC GCC TCC ACC AGT GCA CAC GTT ATA CTG ACT CAA CCG CC-3'
 5'-ACC GCC TCC ACC AGT GCA CAG GCT GTG CTG ACT CAG CCG TC-3'
 5'-ACC GCC TCC ACC AGT GCA CTT AAT TTT ATG CTG ACT CAG CCC CA-3'
 5'-ACC GCC TCC ACC AGT GCA CAG RCT GTG GTG ACY CAG GAG CC-3'
 5'-ACC GCC TCC ACC AGT GCA CWG CCT GTG CTG ACT CAG CCM CC-3'

FIG.2vi